Immunologically silent cancer clone transmission from mother to offspring

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Rare cases of possible materno-fetal transmission of cancer have been recorded over the past 100 years but evidence for a shared cancer clone has been very limited. We provide genetic evidence for mother to offspring transmission, in utero, of a leukemic cell clone. Maternal and infant cancer clones shared the same unique *BCR-ABL1* genomic fusion sequence, indicating a shared, single-cell origin. Microsatellite markers in the infant cancer were all of maternal origin. Additionally, the infant, maternally-derived cancer cells had a major deletion on one copy of chromosome 6p that included deletion of HLA alleles that were not inherited by the infant (i.e., foreign to the infant), suggesting a possible mechanism for immune evasion.

fetus | fusion gene | leukemia

Rare cases of melanoma or hemopoietic malignancies in infants have been recorded that may have been of maternal origin (1). Genetic evidence for a shared, materno-fetal clone of cancer cells has, however, to date, been sparse and based upon limited karotype information (1). Unambiguous attribution of transmission of a cancer clone should be achievable by genetic fingerprinting, the most striking precedent for which is canine transmissible venereal sarcoma (CTVS) in which multiple cases worldwide derive from a single clone (2). Leukemia fusion genes, generated by chromosome translocations, have patient-specific or idiosyncratic genomic sequences at the fusion breakpoints and are frequently early or initiating events (3). They therefore provide stable, specific, and sensitive clonal markers and can unambiguously identify a single-cell origin in different individuals as documented with monozygotic twins with concordant leukemia (4). We report here equivalent genetic scrutiny of a case of concordant maternal and infant ALL/lymphoma with the *BCR-ABL1* fusion gene.

Results

The Mother. The Japanese mother was 28 years old at her child's delivery. No hematological abnormalities had been identified during the pregnancy, and the birth was uncomplicated. Thirty-six days after the delivery, the mother experienced vaginal bleeding. On day 39, she developed fever, and on day 43, bleeding became uncontrollable. Blood showed leukocytosis (206,800/μL) with 97% lymphoblasts, anemia (hemoglobin level: 3.5g/dL), and thrombocytopenia (platelet count: $0.2 \times 10^4/\mu$L). Bone marrow aspiration revealed peroxidase-negative lymphoblasts (99.6% of nucleated cells), which were positive for CD10, CD19, CD20, CD34, TdT, and CD79a. Chromosomal G-banding showed 46,XX,t (9, 22)(q34;q11), and 3.2×10^5 copies/μgRNA of p190-type *BCR-ABL1* mRNA were detected by RT-PCR. She was diagnosed as having B-cell precursor Ph+ ALL (see *SI Text* for clinical treatment).

The Infant. The 11-month-old female offspring of the above mother was admitted to hospital with right cheek swelling. MRI revealed a mass in the cheek (Fig. S1*A*) and a pleural effusion of the lung. There was no lymph node swelling or organomegaly. She was born with normal delivery at 40 weeks, 5 days gestation. There was no history of prenatal abnormalities including intrauterine growth retardation, and she showed normal growth and development until admission.

Laboratory Findings on Infant Samples. Laboratory analyses of the maternal and infant samples was carried out with full ethical approval in accordance with the Declaration of Helsinki (Local ethics approval # CCR2285) and with informed consent of the family (father). Biopsy of the primary jaw tumor showed the presence of small round blue cell tumor with large nucleus/cytoplasm ratio, which diffusely proliferated with partial hyalinization of stroma. A large antibody panel was used to distinguish a sarcoma from lymphoma. LCA, CD10, CD20, CD79a, TdT, CD34, and MIC2 were positive by immunohistochemical staining, and CD3, CD5, CD56, desmin, HHF35, S100, GFAP, chromogranin, and synaptophysin were all negative. No cytogenetic analysis was performed but subsequent FISH analysis revealed positivity for the *BCR-ABL1* gene (Fig. S1*B*).

Cells (48.2%) in the pleural fluid were positive for CD10, CD19, CD34, and HLA-DR and p190-type *BCR-ABL1* chimeric mRNA was detected (9.5×10^4 copies/μgRNA) by quantitative RT-PCR (Q-PCR).

Blood count findings on the infant were as follows; WBC 10,100/μL (segment forms 22%; lymphocytes 72%; monocytes 5%; eosinophil 1%), hemoglobin level 12.5 g/dL, platelet count $38.4 \times 10^4/\mu$L. No blast cells were detected in the cerebrospinal fluid, and there was no morphological evidence of tumor infiltration in bone marrow. Bone marrow aspirates were negative for *BCR-ABL1* chimeric mRNA by Q-PCR. The patient's neoplastic cells had the same immunophenotype and abnormal genotype (*BCR-ABL1* fusion) as her mother's ALL but, in light of the presentation features, she was diagnosed as having B-cell precursor lymphoblastic lymphoma stage III by the St. Jude Staging System (see *SI Text* for clinical treatment of infant).

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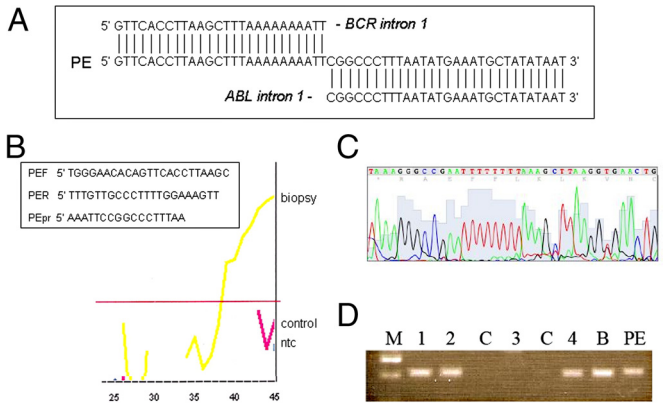

Fig. 1. Characterization of the *BCR-ABL1* fusion gene. (*A*) Comparison of the pleural effusion (PE) *BCR-ABL1* breakpoint DNA sequence with *BCR* intron 1 (NM_004327) and *ABL1* intron 1 (NM_007313) sequences. (*B*) Q-PCR amplification of the *BCR-ABL1* breakpoint in WGA DNA from mother's bone marrow biopsy. The primers and probe shown were chosen to span the PE fusion sequence obtained from the child's PE DNA. (*C*) Reverse strand DNA sequence of DNA from the mother's biopsy showing the same *BCR-ABL1* fusion sequence as found in the child. (*D*) PCR of the *BCR-ABL1* breakpoint in DNA from the neonatal blood spot confirming presence of the *BCR-ABL1* fusion gene. Lanes 1–4: slices from the child's card (1, 2, and 4 positive), C: DNA from control neonatal blood spot, B: mother's marrow biopsy DNA. PE: child's pleural effusion DNA. M: marker.

***BCR-ABL1* Genomic Fusion Sequencing.** We first cloned the *BCR-ABL1* genomic breakpoint region from the infant's pleural effusion (PE) (see *Materials and Methods*). DNA was Whole Genome Amplified (GenomiPhi, GE Healthcare), according to the manufacturer's instructions. The breakpoint was designated as a fusion between *BCR* intron 1 (46110 bp from ATG: NM_004327) and *ABL1* intron 1 (118930 bp from ATG: NM_007313) (Fig. 1*C*).

DNA from the mother's bone marrow was isolated by scraping cells from a formalin fixed paraffin embedded (FFPE) biopsy slide (the only sample available) using Recoverall (Ambion) as suggested by the manufacturer. Fragmented FFPE DNA was then subjected to whole genome amplification (WGA), and 2 μL amplified DNA subjected to 45 cycles Q-PCR with primers designed by Primer 3 software (5) and described in Fig. 1*B* and a FAM-labeled probe that spanned the specific *BCR-ABL1* breakpoint sequence. After successful Q-PCR (Fig. 1*B*), the product was purified and sequenced using the reverse *ABL1* primer. The fusion sequence in the mother's biopsy was verified as identical to that obtained from the pleural effusion of the child (Fig. 1*C*).

The archived neonatal blood spot (Guthrie card) of the infant was screened for the clonotypic *BCR-ABL1* genomic sequence using specific primers and as previously described for other fusion genes (6). Three out of four blood spot slices were positive (Fig. 1*D*), indicating that the cancer clone was present in the blood at birth.

Microsatellite Markers. Short tandem repeat (STR) microsatellite analysis of the DNA extracted from the jaw biopsy showed one predominant population (>95% of alleles) that did not correspond to the DNA profile obtained from the paternal sample (Fig. 2). The STR profile shows that it shared one allele with the patient's germline DNA for all of the 15 STR markers studied which was different from the paternally-inherited alleles, demonstrating that the jaw tumor sample was of maternal origin

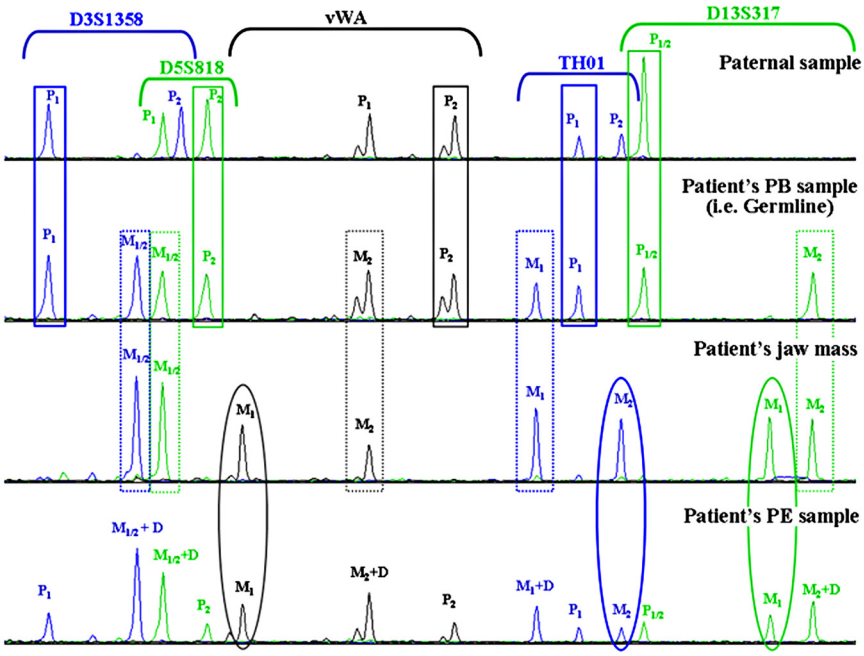

Fig. 2. STR typing for the paternal sample and patient's peripheral blood (PB), lymphoma (from the jaw mass) and pleural effusion samples. Figure shows typing for five out of the 15 STR markers analyzed. The STR profile of patient's jaw mass shows the sharing of one allele with the patient's germline DNA for all markers while the other allele is not present in the paternal DNA, demonstrating that the lymphoma DNA obtained from the jaw mass contained >95% of maternal cells. For each STR marker the Paternal (P1, P2) and Maternal (M1, M2) alleles are indicated. P and M alleles contributing to the patient's germline DNA are contained within solid and dotted rectangles, respectively. The PE sample shows a mixture of different alleles, with some markers showing up to three different alleles (vWA, TH01, and D13S317, contained in ovals), indicating heterozygosity for the maternal genotype on these markers. The remaining maternal alleles contributing to the daughter's genotype in this sample are indicated as M + D. These former markers were used for calculation of the percentage of maternal cells present in the PE sample (≈50–60%) by comparing the areas of the maternal-only peaks (M) versus the paternal peaks (corresponding to the patient's cells). This proportion of maternal cells approximates with the percentage tumor infiltration identified by immunophenotype (48.2%).

Table 1. Loss of non-inherited maternal HLA allele expression in infants 'maternal' cancer cells

	HLA-A	HLA-B	DRB1
Father	A2/A33	B61/B58	DR8/DR13
Mother	A24/**A11**	B60/**B67**	DR9/**DR15**
Patient	A24/A33 2402/3303	B60/B58 4001/5801	DR9/DR13 0901/1302
Patient's jaw mass	A24/-2402/-	B60/-4001/-	DR9/-0901/-

Alleles were serotyped or genotyped (2402/3303, 4001/5801, 0901/1302) (see *SI Text*). Insufficient material was available to genotype parental samples. Alleles in bold are maternal HLA alleles lost from maternal cancer cells transmitted to infant.

(>95% maternal cells). STR analysis of the PE sample showed a mixture of alleles for most STR markers analyzed. These mixtures were identified either because of the presence of three different alleles in some markers, or because those markers with only two alleles presented an imbalance in the ratio of the peak areas (Fig. 2). These findings were consistent with the presence of two genetically different cell populations in the PE specimen–one maternal, one infant offspring (see Fig. 2 legend).

HLA Analysis. Survival of maternal cells in the offspring presumably requires some form of immunological acceptance or tolerance of cells expressing foreign, non-inherited maternal MHC antigens. We serotyped samples from both parents for HLA-A, HLA-B, and DRB1, and both serotyped and genotyped the infant's normal blood and lymphoma cells. The result was that the *BCR-ABL1*-positive jaw tumor had selectively deleted or lost the HLA alleles that were not inherited by the daughter (Table 1). The nature of this genetic lesion in the infant tumor cancer cells was further explored by high resolution SNP arrays. In the absence of normal germline maternal and infant DNA and only small quantities of degraded DNA from the maternal leukemia biopsy, we elected to analyze the infant lymphoma (jaw) sample for genome-wide lack of heterozygosity (LOH) in comparison with pooled normal control DNA. Table 2 summarizes the LOH analysis of the infant lymphoma. Recurrent deletions of *IKZF1* and *CDKN2A/B* have previously been described in *BCR-ABL1* ALL (7), as have deletions of *EBF1* and *RAG1/2* in B lineage childhood ALL (8). In addition to these anticipated oncogenic or 'driver' deletions, we observed a large region of homozygosity on the short (p) arm of chromosome 6 including the whole HLA locus. This loss was accompanied by duplication of the other parental 6p region resulting in uniparental disomy. A large genomic deletion, including the HLA loci, therefore accounts for the loss of maternal HLA alleles.

Discussion

These data unambiguously mark the infant cancer as of maternal origin. Some 17 cases of probable metastasis to the fetus have now been recorded (1, 9, and current report), the first being in 1866, most being either melanoma (#6), a cancer with a notoriously metastatic proclivity, or leukemia/lymphoma (#8). Given the phenotypic features described in these cases, it is likely that they were all, as presumed, of maternal origin rather than coincidental cancers. Genetic markers can unambiguously resolve cellular origins in this context. In three of the reported leukemia/lymphoma cases, the male infant bone marrow contained cells with an XX karyotype (1). Whilst these most probably do reflect maternal leukemia/lymphoma cells, it cannot be excluded that they were non-malignant, normal maternal cells or infant male cells in which the Y chromosome was lost and X was duplicated. These are, individually, not rare events in leukemia (10, 11), although they seldom occur together in one clone. Other prior evidence for a maternal origin was the identification in a case of NK cell lymphoma of a specific chromosome translocation t(X;1)(q22;q12) in the maternal lymphoma and in three metaphases of the infant tumor (12).

Table 2. Loss of heterozygosity analysis of infant tumor lymphoma

Region	LOH	Gene(s)
5p33.3	Loss	*EBF1*
6p25.3–21.1	UPD	*HLA*, and many other genes
7p14.1	Loss	*TCRG*
7p12.2	Loss	*IKZF1*
9p21.3–12	Loss	*MTAP, CDKN2A/B, PAX5*
11p12	Loss	*RAG1/2*
14q11.2	Loss	*TCRA*
14q32.33	Loss	*IGH*
15q22.33	Loss	*SMAD3*
22q11.22	Loss	*IGL*

Loss of heterozygosity analysis of the infant tumor lymphoma in comparison with unpaired control DNA. UPD, uniparental disomy (see *Materials and Methods* for details).

The rarity of materno-fetal transmission of cancer is a testimony to the efficacy of the placental barrier and perhaps to immunosurveillance. In the present case, an additional feature was the selective loss in the infant maternally-derived tumor cells of maternal HLA alleles that were not inherited by the infant (Tables 1 and 2). Loss of HLA would be expected to render the transmitted cancer cells immunologically inert (13). HLA loci encoded cell surface proteins provide the major antigenic targets for allograft recognition and rejection, so it is likely that HLA deletion in this case contributed to successful transmission of the maternal leukemic cells. However, given the large size of the 6p deletion, it is possible that other gene losses could have contributed to the apparent lack of immuno-surveillance. Other unusual situations where cancer cell transmission occurs all appear to involve immunological invisibility (14): inter-monozygotic twin transmission in utero (4), immunosuppressed recipients of cancer-infiltrated donor organs (15), down-regulated MHC antigen expression in venereal sarcoma in dogs (2), and lack of MHC diversity in the Tasmanian devil (*Sarcophilus harrisii*) with transmissible facial tumors (16). In a recent report (17), loss of allorecognition of leukemic cell HLA by T cells, in a transplant context, also occurred by acquired uniparental disomy of chromosome 6p in the leukemic cells, as in the present study. It is possible that materno-feto transfer of cancer cells is more common than is reflected in the frequency of clinically diagnosed cases and that immuno-surveillance is the principal constraint.

Materials and Methods

Detection and Amplification of *BCR-ABL1* Genomic Breakpoints. For detection and amplification of DNA breakpoints, ranging from 300 bp to 12 kbp the Expand Long Template PCR kit (Roche) with System 2 was used, with an annealing temperature of 64 °C. To cover the *BCR* and *ABL1* regions, within which breakpoints can occur, 21 *BCR* forward primers and 20 *ABL1* reverse primers were used in multiplex, combining each *BCR* forward primer with 4 mixes of 5 *ABL1* reverse primers.

The child's genomic breakpoint was initially amplified using *BCR* 3C F (GGGCTCATTTTCACTGGATGGAC) and the *ABL1* D reverse primer mix, and

upon split out PCR, a band was amplified with *BCR* 3C F and *ABL1* 1D R (AGC CAT AAC CAT TCT CCC AAG CA). The breakpoint was confirmed by re-amplification and sequencing of the breakpoint in both the original and WGA amplified patient sample with *BCR* 3C F (GGGCTCATTTTCACTGGATGGAC), and a breakpoint specific *ABL1* reverse primer (TTC AGG GGC CTT GGA TCA GAC TA) determined from sequencing the original cloned product. Forward and reverse primers for blood spot PCR were respectively (GATCCTTTTAAAT-AGGCAAG) and (GTAATGCCAAAAATAACACT).

Fifteen polymorphic STR markers were amplified in the paternal blood DNA and patient's PE and PB DNA samples using the Powerplex-16 system (Promega).

Genome Mapping Analysis. Mapping analysis was performed using 500 ng of tumor DNA from the infant lymphoma. DNA was prepared according to manufacturer's instructions using the GeneChip mapping 500K assay protocol for hybridization to GeneChip Mapping 250K Nsp and Sty arrays (Affymetrix). Briefly, genomic DNA was digested in parallel with restriction endonucleases *Nsp*I and StyI, ligated to an adaptor, and subjected to PCR amplification with adaptor-specific primers. The PCR products were digested with *DNase*I and labeled with a biotinylated nucleotide analog. The labeled DNA fragments were hybridized to the microarray, stained by streptavidin–phycoerythrin conjugates, and washed using the Affymetrix Fluidics Station 450 then scanned with a GeneChip scanner 3000 7G.

Copy Number and LOH Analysis. SNP genotypes were obtained using Affymetrix GCOS software (version 1.4) to obtain raw feature intensity and Affymetrix GTYPE software (version 4.0) using the Dynamic Model algorithm with a call threshold of 0.33 to derive SNP genotypes. The sample was analyzed using CNAG 3.0 (http://plaza.umin.ac.jp/genome), comparing tumor sample with unpaired control DNA to determine copy number and LOH caused by imbalance (18).

HLA Typing. Serotyping was by microdroplet lymphocyte cytotoxicity (19). Genotyping was carried out using a reversed SSO HLA DNA typing method using fluorescent microspheres and a flow analyzer (20).

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1. Alexander A, et al. (2003) Metastatic melanoma in pregnancy: Risk of transplacental metastases in the infant. *J Clin Oncol* 21:2179–2186.
2. Murgia C, Pritchard JK, Kim SY, Fassati A, Weiss RA (2006) Clonal origin and evolution of a transmissible cancer. *Cell* 126:477–487.
3. Greaves MF, Wiemels J (2003) Origins of chromosome translocations in childhood leukaemia. *Nat Rev Cancer* 3:639–649.
4. Greaves MF, Maia AT, Wiemels JL, Ford AM (2003) Leukemia in twins: Lessons in natural history. *Blood* 102:2321–2333.
5. Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. *Bioinformatics Methods and Protocols*: *Methods in Molecular Biology*, eds Krawetz S, Misener S (Humana Press, Totowa, NJ), pp 365–386.
6. Gale KB, et al. (1997) Backtracking leukemia to birth: Identification of clonotypic gene fusion sequences in neonatal blood spots. *Proc Natl Acad Sci USA* 94:13950–13954.
7. Mullighan CG, et al. (2008) BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature* 453:110–114.
8. Mullighan CG, et al. (2007) Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* 446:758–764.
9. Maruko K, Maeda T, Kamitomo M, Hatae M, Sueyoshi K (2004) Transplacental transmission of maternal B-cell lymphoma. *Am J Obstet Gynecol* 191:380–381.
10. Heinonen K, Mahlamäki E, Riikonen P, Meltoranta RL, Rahiala J, Perkkiö M (1999) Acquired X-chromosome aneuploidy in children with acute lymphoblastic leukemia. *Med Pediatr Oncol* 32:360–365.
11. United Kingdom Cancer Cytogenetics Group (UKCCG) (1992) Loss of the Y chromosome from normal and neoplastic bone marrows. *Genes Chromosomes Cancer* 5:83–88.
12. Catlin EA, et al. (1999) Transplacental transmission of natural-killer-cell lymphoma. *N Engl J Med* 341:85–91.
13. Seliger B (2005) Strategies of tumor immune evasion. *BioDrugs* 19:347–354.
14. Greaves M (2000) *Cancer. The Evolutionary Legacy* (Oxford Univ Press, Oxford).
15. Penn I (1991) Donor transmitted disease: Cancer. *Transpl Proc* 23:2629–2631.
16. Siddle HV, et al. (2007) Transmission of a fatal clonal tumor by biting occurs due to depleted MHC diversity in a threatened carnivorous marsupial. *Proc Natl Acad Sci USA* 104:16221–16226.
17. Vago L, et al. (2009) Loss of mismatched HLA in leukemia after stem-cell transplantation. *N Engl J Med* 361:478–488.
18. Nannya Y, et al. (2005) A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. *Cancer Res* 65:6071–6079.
19. Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y (1978) Microdroplet testing for HLA-A, -B, -C, and -D antigens. *Am J Clin Pathol* 69:103–120.
20. Saito K, et al. (2002) A new reversed SSO HLA (Class I and II) DNA typing method using fluorescently labeled microspheres and flow analyzer. *Eur J Immunogenet* 29:173.